

AMENDMENTS TO THE CLAIMS

Claim 1. (Currently Amended) A method ~~Method~~ for the preparation of a strain of evolved micro-organisms for the production of 1,2-propanediol by the metabolism of a simple carbon source, said method ~~comprising~~ comprising:

(a) providing ~~growing~~ an initial bacterial strain comprising deletion of *tpiA* gene and deletion of at least one gene involved in the conversion of methylglyoxal into lactate;

(b) culturing the initial bacterial strain, under selection pressure in an appropriate growth medium comprising a simple carbon source, ~~said initial bacterial strain comprising a deletion of the gene *tpiA* and a deletion of at least one gene involved in the conversion of methylglyoxal (propanal) into lactate~~ source for a time period sufficient to allow an increase in growth;

(c) causing ~~in order to cause~~ evolution, in said initial strain, of one or more genes involved in the biosynthesis pathway from DHAP to methylglyoxal and then to 1,2-propanediol towards evolved genes having an improved "~~1,2-propanediol synthase~~" 1,2-propanediol synthase activity to provide an evolved ~~strain, then~~ strain; and

(d) selecting and isolating the evolved strain of micro-organisms ~~or strains of evolved micro-organisms~~ having an improved "~~1,2-propanediol synthase~~" 1,2-propanediol synthase activity.

Claim 2. (Currently Amended) The method of claim 1, wherein the gene involved in the conversion of methylglyoxal into lactate is selected from the group ~~consisting in~~ consisting of *gloA*, *aldA* and *aldB*.

Claim 3. (Currently Amended) The method of claim 1, wherein the initial strain comprises deletion of ~~the~~ genes *gloA*, *aldA*, and *aldB* ~~and *tpiA*~~.

Claim 4. (Currently Amended) The method of claim 1, wherein the initial strain comprises deletion of ~~the~~ genes *ldhA*, *pflA*, *pflB*, *adhE* and *edd*.

Claim 5. (Currently Amended) The method of claim 1, wherein the initial strain further comprises a pyruvate dehydrogenase complex ~~also contains at least one gene coding for an enzyme that favours the metabolism of pyruvate to acetate.~~

Claim 6. (Currently Amended) The method of ~~claim 1~~ claim 5, wherein the pyruvate dehydrogenase complex ~~enzyme that favours the metabolism of pyruvate into acetate~~ has low sensitivity to inhibition by NADH.

Claims 7 and 8. (Cancelled)

Claim 9. (Currently Amended) The method of ~~claim 6~~ claim 5, wherein the ~~enzyme that favours the metabolism of pyruvate into acetate is an endogenous enzyme~~ pyruvate dehydrogenase complex is endogenous.

Claim 10. (Currently Amended) The method of claim 1, wherein one or more heterologous genes coding for one or more enzymes involved in the conversion of acetyl-CoA and acetate into acetone are introduced into the evolved microorganisms to provide a modified evolved strain.

Claim 11. (Currently Amended) The method of claim 10, wherein ~~one~~ the one or more heterologous gene or genes coding for one or more enzymes involved in the conversion of acetyl-CoA and acetate are from *C. acetobutylicum*.

Claim 12. (Currently Amended) The method of claim 10, wherein the modified evolved strain comprising one or more heterologous genes coding for one or more enzymes involved in the conversion of acetyl-CoA and acetate into acetone is grown under selection pressure in an appropriate growth medium comprising a simple carbon source in order to cause, in said ~~evolved~~ modified evolved strain, the evolution of one or more genes involved in the conversion of acetyl-CoA and acetate to acetone towards an improved "~~acetone synthase~~" acetone synthase activity, the second generation of resulting evolved microorganisms having an improved "~~1,2-propanediol~~

~~synthase~~ 1,2-propanediol synthase activity and an improved ~~"acetone synthase"~~ acetone synthase activity are then selected and isolated.

Claim 13. (Currently Amended) The method of claim 1, wherein the initial strain is a bacterium ~~selected from the group consisting of bacterium, a yeast and a fungus.~~

Claim 14. (Currently Amended) The method of claim 13, wherein the ~~modified strain~~ bacterium is selected from the group consisting of *Escherichia* and *Corynebacterium*.

Claim 15. (Cancelled)

Claim 16. (Currently Amended) An evolved ~~Evolved~~ strain ~~that can be~~ obtained by the method according to ~~any of Claims~~ claim 1

Claim 17. (Currently Amended) The evolved strain ~~Strain~~ according to ~~Claim 16, Claim 35,~~ wherein the evolved strain comprises an *lpd* gene encoding a lipoamide dehydrogenase of the pyruvate dehydrogenase complex, and wherein the ~~in which the gene~~ *lpd* gene has a point mutation whereby alanine 55 is replaced by valine.

Claims 18 to 21. (Cancelled)

Claim 22. (Currently Amended) An initial ~~Initial~~ bacterial strain of a microorganism comprising a deletion of the gene *tpiA* and a deletion of at least one gene involved in the conversion of methylglyoxal (~~propanal~~) into lactate.

Claim 23. (Currently Amended) The strain of claim 22, wherein the gene involved in the conversion of methylglyoxal into lactate is selected among the group ~~consisting in~~ consisting of *gloA*, *aldA* and *aldB*.

Claim 24. (Currently Amended) The ~~method~~ strain of claim 22, wherein the initial strain comprises deletion of the genes *gloA*, *aldA*, and *aldB* ~~and *tpiA*~~.

Claim 25. (Previously Presented) The strain of claim 22, wherein the initial strain comprises deletion of the genes *ldhA*, *pflA*, *pflB*, *adhE* and *edd*.

Claim 26. (Currently Amended) The strain of claim 22, wherein the initial strain also contains ~~at least one gene coding for an enzyme that favours the metabolism of pyruvate to acetate~~ a pyruvate dehydrogenase complex.

Claim 27. (Currently Amended) The strain of claim 22, wherein the ~~enzyme that favours the metabolism of pyruvate into acetate~~ pyruvate dehydrogenase complex has low sensitivity to inhibition by NADH.

Claims 28 and 29. (Cancelled)

Claim 30. (Currently Amended) The strain of claim 22, wherein the strain is a bacterium ~~selected from the group consisting of a bacterium, a yeast and a fungus~~.

Claim 31. (Currently Amended) The strain of claim 30, wherein the bacterium is selected from the group consisting of *Escherichia* and *Corynebacterium*.

Claim 32. (Currently Amended) The evolved strain of claim 16, ~~comprising a deletion of the gene *tpiA* and a deletion of~~ wherein the at least one gene involved in the conversion of methylglyoxal (~~propanal~~) into lactate, ~~selected from the group consisting in~~ lactate is selected from the group consisting of *gloA*, *aldA* and *aldB* to provide a modified evolved strain.

Claim 33. (Currently Amended) The evolved strain of claim 16, comprising deletion of the genes *gloA*, *aldA*, and *aldB* ~~and *tpiA*~~ to provide a modified evolved strain.

Claim 34. (Currently Amended) The evolved strain of claim 16, further comprising a modification, the modification comprising deletion of the genes *ldhA*, *pjlA*, *pjlB*, *adhE* and *edd* to provide a modified evolved strain.

Claim 35. (Currently Amended) The strain of claim 16, ~~comprising at least one gene coding for an enzyme that favours the metabolism of pyruvate to acetate~~ wherein the strain comprises a pyruvate dehydrogenase complex.

Claim 36. (Currently Amended) The strain of ~~claim 36~~ claim 35, wherein the ~~enzyme that favours the metabolism of pyruvate into acetate~~ pyruvate dehydrogenase complex has low sensitivity to inhibition by NADH.

Claims 37 and 38. (Cancelled)

Claim 39. (Currently Amended) The strain of ~~claim 36~~ claim 35, wherein the ~~enzyme that favours the metabolism of pyruvate into acetate is an endogenous enzyme~~ pyruvate dehydrogenase complex is endogenous.

Claim 40. (Previously Presented) The strain of claim 16, comprising one or more heterologous genes coding for one or more enzymes involved in the conversion of acetyl CoA and acetate into acetone.

Claim 41. (Currently Amended) The strain of claim 40, wherein ~~one~~ the one or more heterologous gene or genes coding for one or more enzymes involved in the conversion of acetyl-CoA and acetate is from *C. acetobutylicum*.

Claim 42. (Currently Amended) The strain of claim 16, wherein the strain is a bacterium ~~selected from the group consisting of a bacterium, a yeast and a fungus.~~

Claim 43. (Currently Amended) The strain of claim 16, wherein the bacterium is selected from the group consisting of *Escherichia* and *Corynebacterium*.

Claim 44. (Currently Amended) The strain of claim 17, wherein the strain is a bacterium ~~selected from the group consisting of a bacterium, a yeast and a fungus.~~

Claim 45. (Currently Amended) The strain of claim 17, wherein the bacterium is selected from the group consisting of *Escherichia*, and *Corynebacterium*.

Claim 46. (Currently Amended) An evolved ~~Evolved~~ strain ~~that can be~~ obtained by the method of Claim 10.

Claim 47. (Previously Presented) The evolved strain ~~of Claim 46,~~ according to claim 50, wherein the evolved strain comprises an *lpd* gene encoding a lipoamide dehydrogenase of the pyruvate dehydrogenase complex, and wherein the *lpd* gene ~~in which the gene *lpd*~~ has a point mutation whereby alanine 55 is replaced by valine.

Claim 48. (Currently Amended) The strain of claim 46, wherein the strain is a bacterium ~~selected from the group consisting of a bacterium, a yeast and a fungus.~~

Claim 49. (Previously Presented) The strain of claim 46, wherein the bacterium is selected from the group consisting of *Escherichia* and *Corynebacterium*.

Claim 50. (New) The evolved strain of claim 46, wherein the strain comprises a pyruvate dehydrogenase complex.